

2018-05

# Isolation of Oropouche Virus from Febrile Patient, Ecuador

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<http://hdl.handle.net/10026.1/11733>

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10.3201/eid2405.171569

Emerging Infectious Diseases

Centers for Disease Control and Prevention (CDC)

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Testing of tissues from vacuum aspiration and from chorionic villi sampling revealed that placenta and chorion contained Zika virus RNA. Isolation of Zika virus from the karyotype cell culture confirmed active viral replication in embryonic cells. All the tests performed suggest that the spontaneous abortion in this woman was likely associated with a symptomatic Zika virus infection occurring early in pregnancy. These findings provide further evidence of the association between Zika virus infection early in pregnancy and transplacental infection, as well as embryonic damage, leading to poor pregnancy outcomes (2). Given that embryo loss had probably occurred days before maternal-related symptoms, we hypothesize that spontaneous abortion happened early during maternal viremia. The prolonged viremia in the mother beyond the first week after symptom onset concurs with other recent reports (1,5). However, persistent viremia 3 weeks after pregnancy outcome has not been described previously and underscores the current lack of knowledge regarding the persistence of Zika virus infection. Because we identified Zika virus RNA in placental tissues, our findings reinforce the evidence for early gestational placental tissue as the preferred target for viral tropism (2,4). Finally, although laboratory tests were performed to dismiss other maternal infections (see online Technical Appendix), the attribution of Zika virus as the cause of the spontaneous abortion must be interpreted with caution, because a non-Zika-related etiology cannot be entirely ruled out. Further studies are warranted to investigate the natural history of Zika virus infection in pregnant women.

This work was funded by grant no. PI16/0123. ISCIII-AES-Proyectos de Investigación en Salud, 2016, Government of Spain.

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## Isolation of Oropouche Virus from Febrile Patient, Ecuador

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DOI: <https://doi.org/10.3201/eid2405.171569>

We report identification of an Oropouche virus strain in a febrile patient from Ecuador by using metagenomic sequencing and real-time reverse transcription PCR. Virus was isolated from patient serum by using Vero cells. Phylogenetic analysis of the whole-genome sequence showed the virus to be similar to a strain from Peru.

Oropouche virus (OROV) is a negative-sense, single-stranded RNA virus (family *Bunyaviridae*, genus *Orthobunyaviridae*) with a tripartite genome consisting of large (L), medium (M), and small (S) segments. OROV causes a self-limiting acute febrile illness, Oropouche fever (1). Since its discovery in Trinidad in 1955 (2), >30 outbreaks of OROV have been reported from Brazil, Panama, and Peru, demonstrating the ability of this midgeborne virus to cause epidemics. Approximately 500,000 cases of Oropouche fever have been reported, making OROV one of the most clinically significant orthobunyaviruses (1). Two previous studies reported unconfirmed infections in Ecuador by using

serologic or antigenic evidence (3,4). We describe whole-genome sequencing and virus isolation of OROV in Ecuador.

We collected a blood sample from a consenting 41-year-old male patient in Esmeraldas, Ecuador, who sought treatment in April 2016 after 7 days of fever, headache, joint pain, muscle pain, and nausea. The patient reported that he had been in Esmeraldas for  $\geq 3$  months and had not traveled outside the province during that time. RNA was extracted from plasma of the blood sample and tested at Universidad San Francisco de Quito, Ecuador, and Public Health England, UK, for dengue virus (DENV), chikungunya virus (CHIKV), Zika virus, yellow fever virus, Mayaro virus, *Plasmodium* spp., *Leptospira* spp., and *Rickettsia* spp. by using real-time reverse transcription PCR (rRT-PCR) and conventional RT-PCR assays developed in-house or acquired commercially (Genesig, Primerdesign Ltd., Cambridge, UK). The sample gave borderline results for DENV (quantitation cycle [Cq] 35.3) and CHIKV (Cq 36.6; reference ranges  $\leq 35$  positive, 35–40 borderline,  $>40$  negative) and negative results for the other pathogens.

As an initial screen for other pathogens, we applied unbiased metagenomic sequencing. Analysis of sequencing reads by using Kraken, a system for assigning taxonomic labels to individual reads (5), identified 1% reads (5,016 of 464,444) as specific to OROV. We generated an OROV consensus sequence by mapping reads to a reference sequence, which resulted in coverage of 69% for S, 76% for M, and 79% for L OROV viral RNA segments (online Technical Appendix, <http://wwwnc.cdc.gov/EID/article/24/2/17-1569-Techapp1.pdf>). We classified 1,228 reads as DENV serotype 1, all of which mapped to a single 732-nt region of the DENV-1 reference genome. No reads mapped to CHIKV.

After confirmation of the presence of OROV by using a validated rRT-PCR (6), we attempted to isolate OROV by using Vero and C6/36 cell lines inoculated with the patient's plasma. We confirmed virus replication by detecting increasing OROV RNA over time by using rRT-PCR. We obtained whole-genome sequences by sequencing viral RNA from harvested OROV supernatant; each genome segment was sequenced at average depths of coverage of 55,532 $\times$  for S, 4,954 $\times$  for M, and 5,674 $\times$  for L segments. We deposited sequences in GenBank (online Technical Appendix). Genetic organization was similar to that of other OROV strains: segment lengths 952 nt for S, 4,387 nt for M, and 6,852 nt for L.

Phylogenetic analysis (online Technical Appendix) showed that the virus we isolated, OROV/EC/Esmeraldas/087/2016, was most closely related to a strain isolated from a patient in Peru during 2008 and excluded the possibility of the virus being a reassortant orthobunyavirus, such as Iquitos virus. This finding suggests a potential introduction across the Peru–Ecuador border; however, further investigation is required to understand the origin and incidence of OROV in Ecuador. The known urban OROV

vector, the *Culicoides paraensis* midge, is absent in the Pacific Coast region, including Esmeraldas (S. Zapata, pers. comm., 2017 Aug 31), which raises the question of alternative insect vectors in OROV transmission. *Culex* mosquitoes have previously been implicated as vectors in the OROV urban cycle, notably *C. quinquefasciatus* (1), a species that is widespread throughout South America (7).

DENV and CHIKV rRT-PCR results for this patient were inconclusive. The small proportion of DENV reads in the metagenomic data suggests DENV-1 infection is possible. Using ELISA to detect DENV and CHIKV-specific antibodies may help clarify the results.

It is likely that cases of Oropouche fever go unreported or misdiagnosed. Clinical features of the disease are similar to those of other viral, protozoan, and bacterial diseases previously reported in Ecuador (1,4,8,9). OROV might spread unnoticed across a wide geographic area, as suggested by this unexpected detection. Several studies have successfully documented the use of metagenomic sequencing for virus identification in febrile patients (10); this approach is becoming more practicable as costs decrease, the major benefit being the ability to detect unexpected or novel viral sequences, as evidenced by this detection of OROV.

This work highlights the need for increased surveillance of OROV in Ecuador and effective differential diagnostic assays to distinguish between emerging pathogens sharing common clinical descriptions to those already circulating. To clarify the true prevalence of this disease in Ecuador, the OROV rRT-PCR assay will be used to screen archived and newly collected samples from a cohort of patients seeking treatment for acute undifferentiated febrile illness during 2016–2017.

## Acknowledgments

The authors thank Liana Kafetzopoulou and Kuiama Lewandowski for providing technical support.

This study was approved by the bioethics committee of Universidad San Francisco de Quito. The patients provided written consent indicating that they agreed for their samples to be tested for additional pathogens.

Ms. Wise is a PhD student registered with Plymouth University and funded by Public Health England. Her research interests are emerging viral infections and viral immunology.

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## etymologia

### Oropouche [o'ro-poo"che] Virus

Ronnie Henry and Frederck A. Murphy

In September 1955, a virus was isolated from a 24-year-old forest worker from the community of Vega de Oropouche, near the town of Sangre Grande, on the island of Trinidad (country: Trinidad and Tobago), who presented with fever, backache, and cough, which resolved spontaneously after 3 days. The virus was isolated from the patient's blood by intracranial inoculation of suckling mice at the Trinidad Regional Virus Laboratory. Five years later, the virus was isolated from *Coquillettidia venezuelensis* (Theobald) mosquitoes collected ≈30 miles away in the Bush Bush Forest. The urban vector was later identified as the midge *Culicoides paraensis*, but the sylvatic vector remains unknown. Virus has been isolated from the three-toed sloth, which is believed to be involved in the sylvatic transmission cycle.

The virus was shown to be unique but antigenically related to Simbu virus, which had recently been described from South Africa. It therefore became a member of the large family of bunyaviruses.<sup>1</sup>

The virus, Oropouche virus, named in keeping with the tradition of designating arboviruses by using local geographic names, stems from the name of the village, a nearby swamp (wetland), and river. It derives from an Amerindian word, but the ancient meaning of the word is not clear.

Oropouche virus has since proven to be one of the most common arthropodborne viruses infecting humans in the tropics of the Western Hemisphere. Clinical signs of infection include headache, myalgia, arthralgia, and chills; no deaths have been reported. It has been estimated to have infected more than half a million persons in Brazil alone, and there have also been large outbreaks in Panama and Peru. In keeping with the recent emergence of other arboviruses such as West Nile, chikungunya, and Zika viruses, Oropouche virus is a candidate for possible further urban spread and therefore warrants increased surveillance and diagnostics.

<sup>1</sup>In 2017, the International Committee on Taxonomy of Viruses (ICTV) reclassified the family *Bunyaviridae*, elevating the family to an order. The formal taxonomic placement of Oropouche virus is order *Bunyavirales*; family *Peribunyaviridae*; genus *Orthobunyavirus*; species; *Oropouche orthobunyavirus*.

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DOI: <https://doi.org/10.3201/eid2405.ET2405>